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13. ABSTRACT *(Maximum 200 words)*

Monoclonal murine IgG antibodies against Gp120 from HIV-1 were found to induce significant enhancement of HIV-1 replication in vitro when used at low antibody concentrations.This enhancement was dependent on the presence of FcR:s for IgG and was completely blocked by anti-CD4 monoclonal antibodies.HIV-1 seropositive individuals at a low frequency do produce antibodies reactive with Gp120 and "mimicking" CD4 molecules as evidenced by their reactivity with certain CD4 monoclonal antibodies. It was,however,not possible to show that such "mimicking" antibodies were especially efficient in neutralizing,nor was presence of such antibodies linked to a particular clinical progression behaviour.Presence of antibodies to peptides covering the respective cysteins in the V3 loop of a particular HIV-1 isolate were found using two Italian cohorts to be positively linked to a reduced rate of transmission of virus from mother to child.

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INTRODUCTION

HIV-1 is known to induce profound immunodeficiency and AIDS in the majority of infected individuals.This is so despite the fact that most individuals develop specific anti-HIV immune responses at both the cellular and humoral level.The rate of progression against AIDS in HIV-1 infected individuals is highly variable with a minority maybe even not developing any disease.Likewise, only a fraction of infected mothers will transfer HIV-1 to their children.A major aim of the present project was to try to define a level of humoral immunity under which conditions antibodies against HIV could act in a positive protective way,or alternatively as negative,enhancing antibodies.This meant that the specificity of the antibodies as far as neutralizing or prognostic antibodies was considered an important parameter to analyze.Likewise,monocytes being a cell type that was reported to be susceptible to infection by HIV was also deemed highly relevant to analyze,in particular in relation to the possible danger of virus-enhancing antibodies.The CD4 binding capacity of GP120 was known to be essential for the infectious process.However,it was not known at this time to what degree antibodies blocking the binding of Gp120 to CD4 were positively correlated to good (or bad) prognosis of the infected individual.The V3-region of Gp120 had been established to constitute a major target for neutralizing antibodies in in vitro experiments.It was thus logical to explore if the level of antibodies against the V3-region could be used as prognostic marker ,for instance when determining the risk for maternal transmission of HIV.

BODY

ANALYSIS OF MONOCYTES AS TARGETS FOR HIV INFECTION IN PRESENCE OR ABSENCE OF ANTIBODIES AGAINST HIV.

In our studies we explored whether normal human blood mononuclear cells could be subdivided into HIV resistant or susceptible subgroups depending on phenotypic markers. Separation of T cells and monocytes was produced by SRBC rosetting,percoll gradient separation,adherance and/or FACS sorting using the OKM3 antibody (5).An example of such an experiment is given in Table 1.Our data suggested that under Il-2 lacking-conditions sensitivity of blood monocytes to HIV infection may exceed that of the average T lymphocyte.However,inherent

difficulties to definitely associate the observed reactivities with truely pure monocytes made us move from blood cells into an analysis of human cloned monocytoid cell lines.

For this we used primarily the established human monocytoid cell line U-937 as a system where several stabile cloned lines exist with highly significant phenotypic differences between the clones.In this analysis we could clearly show that susceptibility of various U-937 clones to IIIB infectivity was dramatically different.In Tables 2 and 3 it could thus be shown that there was no association between phagocytic capacity and sensitivity to HIV infection.In contrast,the density of CD4 molecules on the cloned lines seemed to be a decisive factor where higher density/ frequency of positive cells correlated positively with more rapid progression in vitro of HIV infection.We did thus conclude that the actual concentration of CD4 on the surface could be a more decisive factor determining relative HIV susceptibility of a cell than whether the cell was a T cell or a monocyte.Presence of FcR:s for human IgG was routinely found on human monocytes/monocytoid cell lines but failed in the absence of antibodies against HIV to play any measurable role with regard to HIV sensitivity.

We next explored whether presence of HIV antibodies at certain concentrations might have an enhancing effect with regard to HIV replication using as targets U-937,clone 2,cells.These cells are quite sensitive to HIV infection but were not the most susceptible U-937 cells.However,clone 2 cells are high expressors of FcR:s and express efficient phagocytosis in the presence of IgG-antigen complexes.Using varying doses of virus and dilution of serum from HIV-infected donors (lacking functional virus by containing HIV antibodies)we next tested whether fresh PBL:s,U.937 clone 2 cells, K562 (FcRIgG+human erythromyeloid leukemia cells lacking CD4) or Raji (FcRIgG+ Burkitt-lymphoma cells lacking CD4)could be infected with IIIB virus in vitro.An example of the results obtained in such experiments is given in Table 4.In the CD4 negative cells (=K562 and Raji)no growth of IIIB HIV was found regardless of whether the virus suspension contained HIV antibodies or not.In contrast using either PBL:s or U-937 clone 2 cells the level of HIV infection obtained in normal human serum was frequently reduced at high concentrations of serum from HIV+ donors whereas at relatively high dilutions (=low concentration of antibodies) a more rapid growth of the virus and at lower initial viral doses was

observed.The relative degree of enhancement of HIV infection was, however deemed low in comparison to more classical viral enhancement systems such as dengue fever virus.

To further refine the analysis of HIV enhancement by antibodies we turned to the use of monoclonal antibodies at limiting dilutions as well as using limiting dilutions of virus (8).The aim of these studies was twofold:a)Could it be safely defined that the enhancing factor(s) in the sera from HIV+ donors indeed were antibodies and if so of what kind of specificity and b)would antibodies at a given concentration and relative ratio to virus not only enhance the rate of viral production but also reduce the TC-ID50 level.The latter question was considered relevant as to the possible danger that i.e. under certain future vaccine trials levels/and or specificities of antibodies may be reached with harmful effect as to reducing the infectious dose of HIV in vivo.Results of such studies are exemplified in Tables 5 and 6.It could be clearly shown that murine IgG monoclonal antibodies against Gp120 could have a similar enhancing effect on HIV replication as polyclonal human immune serum at certain low concentrations of antibody.The factor of enhancement noticed with the monoclonal antibody was in some cases quite dramatic.Intriguingly,anti-CD4 monoclonal antibodies did completely block the enhancing capacity of the monoclonal Gp120 antibodies.

Our conclusions from the studies performed under this project are that antibodies against HIV can perform as mostly modestly enhancing antibodies in vitro at certain,normally quite low concentrations.It was also possible to demonstrate that the same antibodies at high concentration could act as neutralizing anti - bodies whereas at low concentrations they would cause viral enhancement.Monocytes could be shown to act as target cells for the enhancing antibodies indicating FcR:s for Ig as key molecules in such enhancement.In the present studies binding of IgG-coated HIV particles to the FcR-positive cells was,however,not enough for viral infection to take place.In our studies,a parallel presence of CD4 molecules on such FcR-positive cells thus seemed an absolute prerequisite for HIV infection.

PRESENCE AND FUNCTION OF ANTIBODIES AGAINST GP120 LOOKING LIKE CD4 MOLECULES

Human HIV-seropositive sera contain normally high titers of antibodies that block Gp120 from binding to CD4 (1).One would expect that a fraction of such Gp120 antibodies would display molecular mimicry to the CD4 molecules,that is behave like CD4 molecules in certain serological (and possibly) biological tests. An unusual functional consequence of such antibodies could be considered,for instance as exceptionally efficient antibodies in blocking virus infectivity.There exist a battery of well defined murine monoclonal antibodies directed against human CD4, reacting with determinants involved in or separated from binding of human CD4 to Gp120.We accordingly explored the possible presence of antibodies in human normal or HIV-seropositive sera with ability to block the binding of a particular monoclonal antibody,T4.2 to CD4.T4.2 is highly efficacious in blocking HIV infectivity via binding to CD4.As a control we included another murine anti-human CD4 monoclonal antibody,OKT4,with no capacity to block Gp120 binding and thus HIV infectivity.It was possible to define that certain sera (3/58)from HIV-1 infected individuals indeed contain antibodies competing with T4.2 but not OKT4 antibodies in the binding with cellsurface CD4 or recombinant CD4 molecules (15).We could show that the active factor in the sera from the HIV infected individuals contained or was associated with IgG as shown by its binding to protein A-Sepharose.Immunosorbants made of T4.2-Sepharose was also absorbing away the inhibitory molecules and when elution was made at low pH from such columns human IgG molecules were eluted with binding capacity to Gp120=the molecules in these HIV-1 seropositive sera were IgG antibodies with a dual specificity (=molecular mimicry of the part of CD4 that binds to Gp120 and with binding capacity to Gp120 as well).The binding data suggested that such antibodies might have an unusual capacity in neutralizing HIV.However,we were unsuccessful in neutralizing HIV in vitro using the mimicking human IgG molecules eluted from the T4.2-Sepharose immunosorbant.No convincing linkage was found between presence of these CD4-like antibodies and good (or bad) clinical prognosis when we subsequently extended these studies to

look for clinical outcome in antibody+ or - individuals .We also extended our search for these kind of antibodies in a very large panel of normal and HIV-1 seropositive sera.It was then possible to show that there exist in very rare normal sera "natural" antibodies with a similar/same specificity as is found but at a higher frequency in sera from HIV-1 infected individuals. Another monoclonal antibody reacting with a different epitope within the Gp120 binding region of CD4 than T4.2 was also used to detect additional subsets of CD4 mimicking antibodies,again found at the highest frequency in HIV+ sera but again without correlation to clinical rate of progression.

INDICATIONS THAT PRESENCE OF HIGH TITERS OF ANTIBODIES AGAINST V3 MAY CORRELATE WITH PROTECTION AGAINST MATERNAL TRANSMISSION OF HIV-1.

Transmission of HIV-1 from mother to child represents a unique system for the analysis of potential positive/negative HIV serological reactions. Only a fraction of the children born to HIV-1 infected mothers will become infected with the virus.One reason for this could be that non-transmitting and transmitting mothers differ with regard to quantity/quality of antibodies against HIV.A finding of a positive correlation between the presence of certain antibodies of a given specificity and transmission/protection could provide significant leads as to in vivo protective antibodies and their respective epitopes.

In order to scan for such potential epitopes we were using 15 aminoacid long peptides produced from the immunodominant regions of env,pol and gag of HIV-1 in ELISA tests.HTLV-IIIB isolate sequences were used in constructing the peptides (9).Sera were obtained from two cohorts,both from Italy.The first cohort consisted of infants born to HIV infected mothers and whose HIV status was known at the time of analysis.Sera were obtained during the first 6 months of age (=predominantly maternal antibodies). The second cohort consisted of sera from infected mothers whose children had been monitored for a mean period of 15 months after delivery.Table 7 shows the peptides used in this study.Note in particular the Gp120 peptides denoted C51,53,57,58 which are derived from the immunodominant V3 region.When in the first cohort sera from the children were analyzed there was a suggested

overrepresentation of antibody reactivity in the sera of uninfected children reactive with peptide Gp120/C57.This was confirmed in the second cohort as shown in Table 8 where now reactivity against the C57 peptide was found 9/13 non-transmitting mothers compared to 1/8 of the transmittors (p=0.0162 by Fisher´s exact test).Antibodies against a second peptide,again in the V3 region but now in the aminoterminal end of the V3 loop (C57 is as the carboxyterminal part of the V3 loop),C51,was also found to be significantly overrepresented in sera from non-transmitting mothers (p=0.0375 by Fisher´s exact test).There were no significant differences in the reactivity to the other peptides between non-transmitting and transmitting mothers.Our data would thus suggest that at least in certain cohorts V3 peptide sequences covering the cysteine-containing parts of that loop may contain epitope against which antibody reactivity has a positive correlation to prognosis (=transmission of HIV-1 or not from mother to child).It is,however,clear that these findings need repetition using other cohorts and preferentially using sequences derived from the autologous virus isolate.

PRODUCTION AND ANALYSIS OF GP120 IN RELATION TO CD4 BINDING CAPACITY

In order to analyze the CD4-binding capacity of Gp120 and the fine specificity of antibodies towards Gp120 blocking CD4 binding we needed to have available native Gp120 in sufficient quantities. We thus constructed a system for transient expression of Gp120 in a transfection system using a vector with a SV40 late replacement vector (2).We could also show that certain amino acids like cystein 402 was essential for binding to CD4 and elimination of this cystein did also lead to rapid intracellular degradation (7).In attempts to define if the CD4 binding domain could be identified in more detail we used two approaches, proteolytic degradation of native Gp120 analyzing the fragments for CD4 binding capacity (4) or determining by various vectors where limited parts of the Gp120 gene had been inserted (11) if a particular CD4 binding region could be determined.It was possible to define using this protocol that a fragment of Gp120 from position to when expressed in a bovine papilloma virus still retained CD4 binding capacity.

CONCLUSIONS

Antibodies against HIV can appear in many forms and functions. Our studies have shown that antibodies against the env protein of HIV can act not only as neutralizing but also as enhancing antibodies.It would now be of importance to verify whether such an in vitro enhancing capacity has an in vivo counterpart.Likewise, antibodies mimicking CD4 and thus considered as potentially especially efficient neutralizing antibodies were found in some HIV-infected individuals.However,it was not possible to delineate any special biological functions to these antibodies,nor was it possible to correlate presence of such antibodies to a particular positive,clinical course.Searching for clinically relevant antibodies, associated with protection against maternal transmission of HIV-1,there seem to exist a possibility of linking the presence of certain antibodies against Gp120 epitopes/peptides with reduction of transmission.This needs further confirmation in additional cohorts and using different peptides or functional assays but would,if confirmed,open up for the possibilities of intervention during late pregnancy with high titered HIV-immunoglobulin .Our search for obtaining a freely dissected CD4-binding region of Gp120 may be futile but should continue,as it may enhance the likelihood of obtaining crystals of such fragments of Gp120 in a complexed form with CD4.

Table 1. Subsets of Human Blood Mononuclear Cells and Sensitivity to Infection with a Given HIV Isolate

Cell subset	% Fluorescent cells with marker:					ConA[a] (cpm)	HIV-RT[b] (cpm)
	M3	CD3	CD4	CD8	HLA-DR		
Unseparated	8	75	50	28	21	234,040	13,500
Large T	<1	96	59	54	<1	7,420	18,300
Medium T	<1	98	70	48	2	6,436	6,800
Small T	<1	99	74	53	<1	420	8,700
Adherent	40	28	ND[c]	ND	ND	47,620	44,900
$M3^+$	>99	ND	ND	ND	ND	1,440	12,900
$M3^-$	2	ND	ND	ND	ND	49,856	11,900

Table 2. Lack of Correlation between Phagocytic and Nonphagocytic Capacity in Human Monocytes and Susceptibility to HIV in the Absence of Anti-HIV Antibodies

Cells	% Phagocytosis[a]	% ADCC[b]	M3 marker[c]	ConA response[d]	HIV proliferation[e]
Blood monocytes					
$M3^+$	11.5 (0.8)	8	>99%	1	12.4 (9.4–19.0)
$M3^-$	1.0 (1.0)	45	3%	26	11.9 (9.3–14.3)
U937 subclones					
Parental line	0	71	0%		0.7
Clone 1	31	0	100%		0.9
Clone 2	25	2	100%		223.5
Clone 16	0	81	0%		729.6

Table 3. Phenotype and HIV Sensitivity of U937 Parental Line and Clones

Cell	% Highly positive cells with:			Lysosomal enzyme level	Day postinfection when HIV sensitivity[a] detected
	HLA-DR	CD4	FcR		
Parental	10–20	<10	35	Low	48
Clone 1	>90	50–60	100	High	20
Clone 2	>90	60–70	100	High	10
Clone 16	10–20	>95	15	Low	6

Table 4. Preliminary Studies on the Impact of HIV-Seropositive Sera on HIV Replication in Various Cell Types

Cell type[a]	Virus dose	HIV serum dilution in 10% NHS[b]	HIV-RT and CPE on day[c]							
			4	6	7	11	13	14	17	21
PBL	1	10% NHS		11.5			4.0			
PBL	1	10% HIV$^+$ serum		11.0			4.6			
PBL	1	1% HIV$^+$ in 10% NHS		11.4			9.6			
PBL	1	0.1% HIV$^+$ in 10% NHS		59.1			All dead			
PBL	1:10	10% NHS		<1			4.4			
PBL	1:10	10% HIV$^+$ serum		<1			7.7			
PBL	1:10	1% HIV$^+$ in 10% NHS		<1			21.6			
PBL	1:10	0.1% HIV$^+$ in 10% NHS		1.4			All dead			
K562	1	10% NHS		2.2			<1			
K562	1	10% HIV$^+$ serum		<1			<1			
K562	1	1% HIV$^+$ in 10% NHS		1.1			<1			
K562	1	0.1% HIV$^+$ in 10% NHS		1.4			<1			
Raji	1	10% NHS		2.8			<1			
Raji	1	10% HIV$^+$ serum		4.3			3.1			
Raji	1	1% HIV$^+$ in 10% NHS		3.1			1.0			
Raji	1	0.1% HIV$^+$ in 10% NHS		5.2			1.4			
U937, clone 2		10% NHS	<1, −		2, ±	21, +		16, ++	34, ++	45, ++
U937, clone 2		1:5	<1, −		<1, ±	<1, +		<1, ±	1, −	<1, −
U937, clone 2		1:25	<1, −		<1, ±	10, ±		18, ++	41, ++	65, ++
U937, clone 2		1:125	<1, −		2, +	15, +		12, ++	131, ++	109, ++
U937, clone 2		1:615	<1, ±		3, +	20, +		15, ++	90, ++	140, ++
U937, clone 2		1:3,125	<1, ±		9, +	25, +		16, ++	56, ++	106, ++
U937, clone 2		1:15,625	<1, −		6, +	14, +		4, ++	98, ++	89, ++

Table 5. Effect of mouse ascites fluids containing monoclonal anti-gp120 antibodies on infection of U937-2 cells by HTLV-IIIB

Virus (RT)	10% NHS Ascites (dilution)	Ascites 1 CPE*	Ascites 1 RT†	Ascites 2 CPE	Ascites 2 RT
—	1:200	−	0.9	−	0.8
100	1:200	−	0.8	−	1.1
100	1:2000	±	0.9	±	1.1
100	1:20,000	−	0.7	+	49.7
100	1:200,000	+ +	437.4	−	0.2
100		±	1.2	−	0.5

Table 6. Effect of anti-CD4 antibody (T4,2) on the enhancement of HIV-infection of U937-2 cells by mouse ascites fluid containing anti-gp120 antibody

Cells	Virus (RT)	Ascites (dilution)	Days after infection 8	12	16
U937-2					
Untreated	—	1:20,000	0.7*	0.7	0.6
	50	1:20,000	13.3	228.7	33.0
	50	1:200,000	10.9	133.5	33.9
	50	—	4.2	27.8	19.4
Anti-CD4-treated	—	1:20,000	1.1	0.7	0.8
	50	1:20,000	0.8	1.1	0.8
	50	1:200,000	0.9	0.6	2.1
	50	—	0.9	0.8	0.9
HUT-78					
Untreated	50	—	1.2	29.6	14.3
Anti-CD4-treated	50	—	0.7	0.5	1.1

Table 7. Sequences of synthetic HIV-1 peptides assayed

Peptide	Location		Sequence
	Gene	aa residues	
gp120/C51	*env*	294–308	INCTRPNNNTRKSIR
gp120/C53	*env*	304–318	RKSIRIQRGPGRAFV
gp120/C57	*env*	324–338	GNMRQAHCNISRAKW
gp120/C58	*env*	329–343	AHCNISRAKWNNTLK
gp120/C90	*env*	489–503	VKIEPLGVAPTKAKR
SP22 gp120	*env*	497–511	APTKAKRRYYQREKR
p17/9	*gag*	13–27	LNRWEKIRLRPGGKK
p24/56	*gag*	248–262	GWMTNNPPIPVGEIY
p24/59	*gag*	63–77	KRWIILGLNKIVRMY
p15/94	*gag*	438–452	WPSYKGRPGNFLQSR
pol/B98	*pol*	944–958	DSRNPLWKGPAKLLW

Table 8. HIV-seropositive mothers of an uninfected or an infected child: Reactivity to HIV synthetic peptides and recombinant proteins.

Peptide tested	No. of sera with reactive antibodies/ total no. of sera tested	
	MUC	MIC
gp120/C51	8/13*	1/8*
gp120/C53	6/13	2/8
gp120/C57	9/13†	1/8†
gp120/C58	6/13	1/8
PB1	10/13	3/8
gp120/C90	5/7	4/4
SP22	6/7	3/4
p121	7/7	4/4
pENV9	7/7	4/4
p17/9	6/7	2/4
p24/56	4/7	2/4
p24/59	3/7	3/4
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